Lab-Based Surveillance of *Shiga*-toxin producing *Escherichia coli* in Arizona, 2008

Reporting positive laboratory tests for *Shiga*-toxin producing *Escherichia coli* (STEC) to ADHS is required under Arizona Administrative Code R9-6-204. All commercial, diagnostic and hospital laboratories must submit reports of positive tests to the ADHS Office of Infectious Disease Services and send the isolate to the Arizona State Public Health Laboratory (ASL) for further confirmation and serotyping. In 2008, 70 confirmed and probable cases of *Shiga*-toxin producing *Escherichia coli* were reported to ADHS.

Basic information about the patient's sex, age, and address is included in the laboratory reports. State and local public health departments in Arizona collect additional information about clinical illness and risk factor exposures using a standardized questionnaire. These epidemiological data, along with laboratory information from the testing performed at ASL, provide the basis for identifying STEC clusters or cases that may be of public health concern. Of the 70 STEC cases reported in 2008, 57% are female, with a median age of 4 years with 53% of the cases occurring in children 5 years of age or less.

The Arizona State Public Health Laboratory is able to provide specialized serotyping and strain typing of all STEC isolates and broths submitted. The isolates sent to ASL are serotyped using a rapid test that identifies the presence of Shiga toxin. The broths sent to ASL are serotyped using a combination of the rapid test and polymerase chain reaction (PCR) and are tested for Shiga toxin 1 (*stx*1), Shiga toxin 2(*stx*2), *Escherichia coli* attaching and effacing (*eae*) gene, and the enterohaemorrhagic *Escherichia coli* haemolysin (e-*hly*) gene. For both isolates and broths, if a Shiga-toxin producing isolate is identified, ASL will confirm the presence of O157:H7 and will send the isolate to the Center for Disease Control and Prevention (CDC) if they are unable to confirm the serotype. Apart from serotyping, all STEC O157 isolates received are also genetically fingerprinted by using pulsed-field gel electrophoresis (PFGE), using *Xba* I as the restriction enzyme. The fingerprints generated are processed using Bionumerics software. PFGE matches provide further evidence that the organisms involved in separate cases are similar. Most clinical laboratories are able to identify the presence of Shiga toxin-producing organisms or to culture *E. coli*, but the testing listed above is unique to public health laboratories and can provide information important to public health investigations.

Of the 70 cases of STEC reported to ADHS, isolates were received at ASL for 56 (80%). The majority of the submissions were from commercial and diagnostic laboratories (37, 66%); 19 (34%) were from hospital laboratories. Of the fourteen isolates that were reported to ADHS but were typed at a separate laboratory (either the commercial laboratory or CDC); information is known about O typing for a total of 7(50%) cases.

The 63 isolates identified and serotyped were predominantly *E. coli* O157:H7 (N=23/63). (Table 1) Fifteen isolates submitted to ASL had evidence of Shiga-toxin producing organism detected but the organism could not be isolated or serotyped. The distribution of STEC serotyped by ASL, CDC and the external laboratories is shown in Figure 1. In 2008, 24 STEC O157 isolates were tested with PFGE; 21 of these isolates were assigned a banding pattern. PFGE cluster analysis identified four small clusters of patterns: EXHX01.0047 (N=7), EXHX01.4462 (N=2), EXHX01.3532 (N=2) and EXHX01.0087 (N=2). There did not seem to be a common source for

the few patients in the small clusters that were identified by PFGE. These clusters had onset dates of infection that were spread out over a large timeframe. It is important to note that while PFGE can be used to detect outbreak clusters, it can also identify endemic strains within a region. The distribution of the banding patterns can be observed in Figure 3.

Table 1: Shiga-toxin producing* Escherichia coli *isolates identified and serotyped at ASL, CDC or external commercial laboratory

Serotype	Number of isolates	Percent of total (%)
***E. coli* O157**	**27**	**38.6**
E.coli O157:H7	23	
E.coli O157:Nonmotile	2	
E.coli O157:H type-unknown	2	
***E.coli* O26**	**12**	**17.1**
E.coli O26:H11	9	
E.coli O26:H-type unknown	3	
***E.coli* O91**	**1**	**1.4**
E.coli O91:H14	1	
***E.coli* O111**	**5**	**7.14**
E.coli O111:H8	1	
E.coli O111:Nonmotile	4	
***E.coli* O103**	**1**	**1.4**
E.coli O103:H2	1	
***E.coli* O109**	**1**	**1.4**
E.coli O109:H21	1	
***E.coli* O121**	**1**	**1.4**
E.coli O121:H-type unknown	1	
Evidence of STEC, unable to isolate for confirmation	**15**	**21.4**
Unknown serotype	**7**	**10**
Totals	**70**	

Figure 1: Percent distribution of Shiga-toxin producing* Escherichia coli *isolates reported in Arizona, by O type, 2008

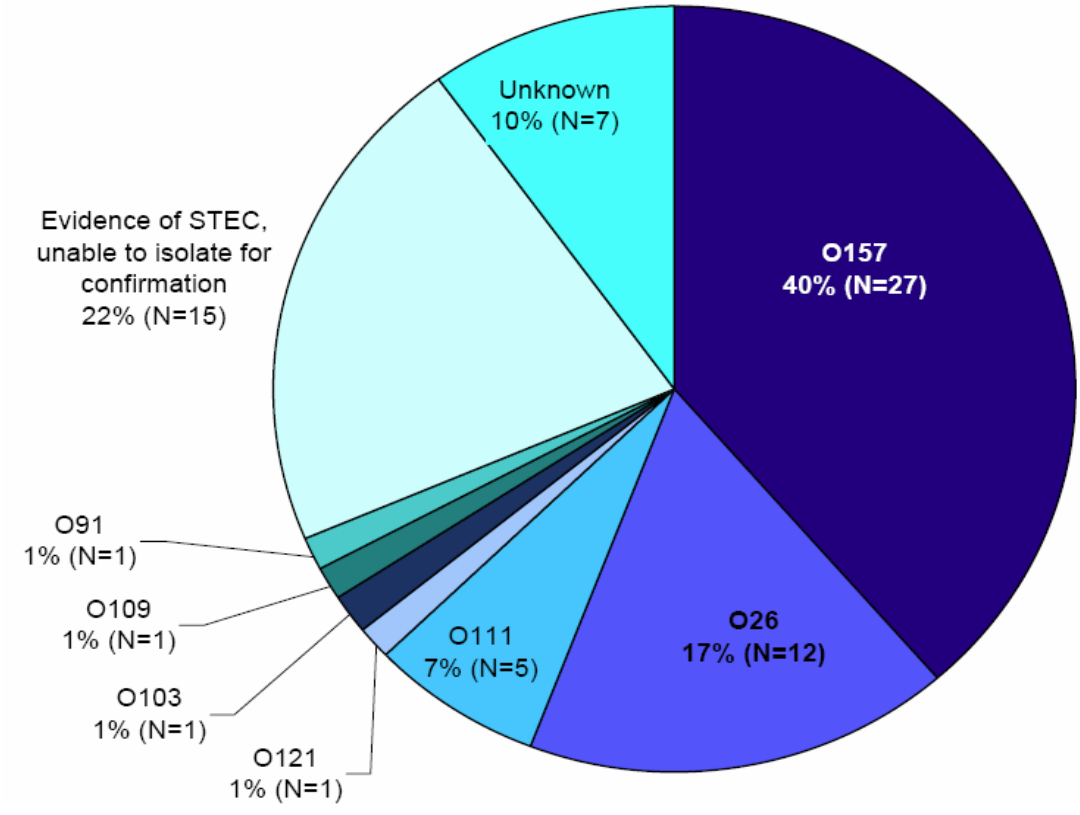
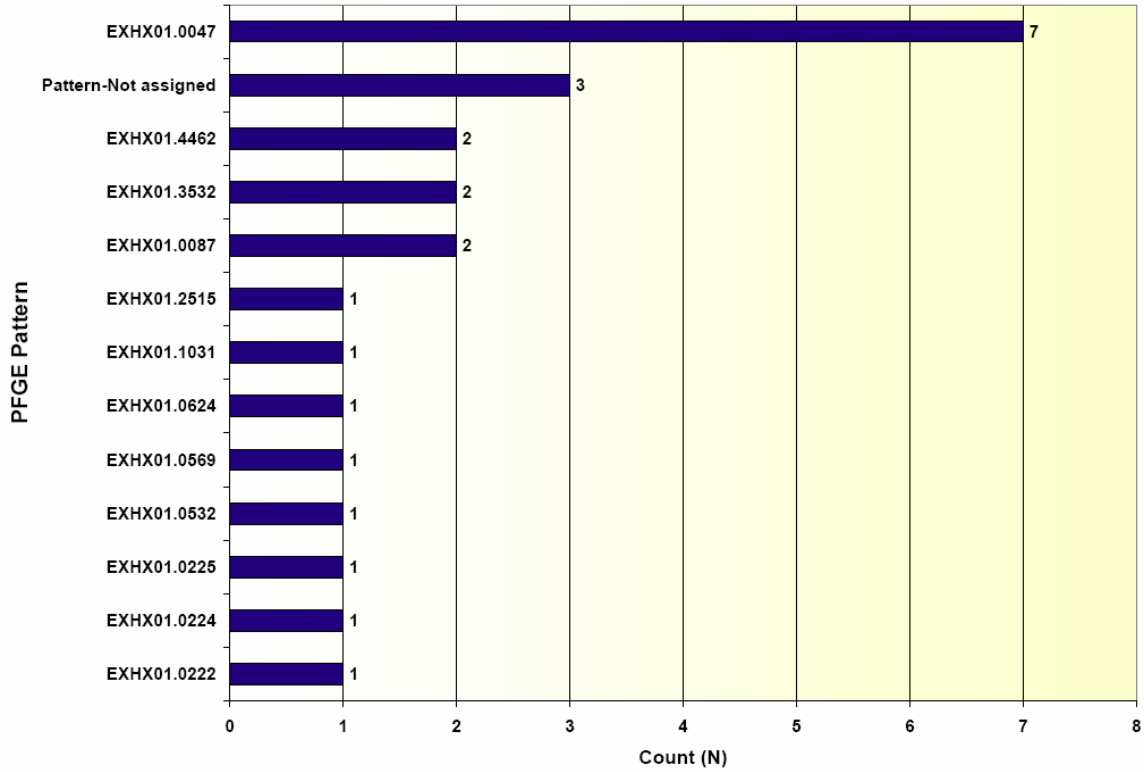


***Figure 3: Pulsed-field gel Electrophoresis pattern distribution of* STEC O157 isolates, Arizona 2008**



In assessing the epidemiological data, 50 (71%) standardized questionnaires were completed for the 70 cases of reported Shiga-toxin producing *Escherichia coli*. Of the 50 cases for which questionnaires were available, diarrhea was present in 96% of cases. Twenty-five percent were hospitalized; none were employed as food handlers. Contact with farm animals or manure was reported by 10 (14%), consumption of raw or undercooked beef by 35 (50%), consumption of raw milk or cheese made from raw milk by 9 (13%), and contact with a symptomatic individual by 18 (26%). Previous studies examining risk factors for sporadic cases of STEC have identified lack of hand-washing by food preparers, lack of washing of work surfaces contaminated with raw ground beef, consumption of hamburgers, visit to a farm, occupational contact with farm animals, and a household contact with diarrheal illness (van Duynhoven *et al*., 2002). Two cases reported developing hemolytic uremic syndrome (HUS); both cases were infected with STEC O157, which is consistent with the literature indicating that STEC O157 is the major cause of diarrhea-associated HUS (Griffin *et al.*,1991). Other than the HUS finding, no associations were noted between the risk factors listed and the O-types found.

The laboratory-based surveillance of *Shiga*-toxin producing *Escherichia coli* is greatly influenced by the likelihood of a patient seeking medical attention, the collection of patient samples for STEC testing, and the submission of isolates to the state laboratory for STEC identification and serotyping. Nevertheless, the seriousness of *Shiga*-toxin producing *Escherichia coli* infections and the potential for outbreaks warrant the need for ongoing laboratory testing and surveillance in Arizona. ADHS will continue to monitor these reports and results, and analyze the distribution of O-types within the state.

References:

1. Griffin PM, Tauxe RV. *The epidemiology of infections caused by Escherichia coli O157:H7, other enterohaemorrhagic E. coli, and the associated hemolytic uremic syndrome*. Epidemiol Rev 1991;13:60-98.

2. van Duynhoven Y., de Jager C.. *Enhanced Laboratory-Based Surveillance of Shiga-Toxin-Producing Escherichia coli O157 in The Netherlands*. European Journal of Clinical Microbiology & Infectious Diseases 2002; Vol 21, 7: 513-522